Selective Adsorption of Biopolymers on Zeolites

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Abstract: Zeolites adsorb biopolymers on their surface and may be suitable as a new type of chromatographic carrier material for proteins, nucleic acids, and their conjugates. We report here various parameters that influence the adsorption of biopolymers on synthesized zeolites with regard to the Si/Al_2 ratio and three-dimensional structure. There are three physicochemical principles that may underly the adsorption: 1) below the isoelectric point (pI), mainly Coulombic attraction similar to ion-exchange chromatography; 2) at pI, hydrophobic interactions (a kind of van der Waals attraction) plus the three-dimensional mesopore structure; and 3) above pI, the sum of the Coulombic repulsion and attraction forces, such as the hydrophobic interaction, and also substitution reaction of water on the Al molecule with a protein amino-base. At high Si/Al_2 ratio in the presence of a small amount of Al and with mesopores between the zeolite particles, maximal adsorption was seen at pI and was suggested to be dependent on the number of hydrophobic interaction points on the mesopores, and their morphology. The application of zeolites to biochemistry and biotechnology is also discussed.

Keywords: adsorption · nucleic acids · proteins · zeolites

Introduction

The purpose of this study was to find new chromatographic carriers for the separation of biopolymers under extreme conditions unlikely to occur in conventional biochemistry; for example, fractionation of biopolymers at temperatures below 0 °C under high pressure and fractionation of conjugated proteins and/or glycoconjugates that precipitate at the point of neutralization. Many proteins that are released and solubilized from biological structural matrices become very unstable and, consequently, are essentially irrelevant from a biochemical perspective. The biochemical solvent is water, and high pressure can maintain this solvent in the liquid state at temperatures below 0 °C. Unstable proteins may survive under such conditions, and carriers must be ultrastable and tough. The microscopically visible structures within cells and/or biological structural matrices are mostly comprised of conjugated proteins and glycoconjugates, and most of these are water-insoluble in that they cannot be separated into their chemical components in water. The development of methods for chromatography of unstable proteins at temperatures below 0 °C and direct fractionation of conjugated proteins and glycoconjugates in the insoluble state could have far-reaching consequences in the field of biochemistry.

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New adsorption carriers based on principles different from those underlying conventional chromatography methods must therefore be investigated. Previously, we reported a new chromatographic carrier made up of silica, a new type of optically active organic–inorganic composite.[1, 2] In the present study, we found that zeolites are much better carriers than this composite. During the course of our experiments, the results of a few preliminary studies attempting to purify proteins by using zeolite Y appeared in the literature.[3–6] In these studies, adsorption was reported to be dependent on pH value and ionic strength, and was shown to occur at the isoelectric point (pI), the pH value in solution at which the sum of charges on the protein is zero, as the aggregate lost its electrical charge. These reports were quite interesting for the reasons given above, but the conclusions were based only on the results of preliminary experiments analyzing the adsorption of a few proteins to zeolite Y. Very little is known about the principles underlying this adsorption. In the present study, therefore, the physicochemical principles underlying the adsorption were systematically investigated by using many species of zeolites and various biopolymers. Since zeolites can be synthesized from aqueous basic aluminosilicate precursor gels under hydrothermal conditions at elevated temperatures,[7–9] we analyzed various parameters that influence the adsorption of proteins and nucleic acids on synthesized

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zeolites with regard to Si/Al_2 ratio and morphology, and we also examined why proteins were selectively adsorbed to zeolites. Our results indicated that physicochemical principle was more complex than the previous explanation that adsorption occurred at pI as the aggregate lost its charge.

Results and Discussion

Zeolites are crystalline porous solids, with pores and channel systems in the molecular size range of 0.3 to 3 nm; they are also tectosilicates that consist of corner-sharing AlO_4 and SiO_4 tetrahedra. These physicochemical characteristics are thought to be the basis for their immense importance in catalysis, separation, and ion exchange.[7–14] The Si/Al_2 ratio of zeolites can be varied either during synthesis or postsynthetically. The Si/Al_2 ratio is also used to denote the hydrophobicity of zeolites, with higher ratios indicating a higher degree of hydrophobicity and lower ion-exchange capacity. The morphology of the structure can also be varied.

The zeolites used in this study were chosen by considering the molecular sizes of pores and the Si/Al_2 ratios. Proteins with varied isoelectric points (pI) and molecular weights (MW), and nucleic acids were chosen as chromatographic markers. The zeolite species tested included zeolite Y, zeolite USY, zeolite beta (Na-BEA), ferrierite (FER), zeolite L (K-LTL), and mordenite (MOR). The Si/Al_2 ratios and the pore sizes of zeolites used are shown in Table 1. Zeolite Y included proton type (H-Y) and Na type (Na-Y). Zeolite USY included three species with different Si/Al_2 ratios (6.3, 10.7, and 13.7), while both zeolites FER and MOR were each composed of two species, K-FER and H-FER, and H-MOR and Na-MOR, respectively. Zeolite X was not used because its crystal structure is the same as that of Y except for the higher Si/Al_2 ratio. Zeolite A was also not used because it is water soluble. In general, zeolites have a low Si/Al_2 ratio and therefore a high ion-exchange capacity. If the Al content and, thus, the ion-exchange capacity, of a zeolite is reduced, it becomes more hydrophobic or organophilic in its adsorptive characteristics. Y zeolites with high Si/Al_2 ratios are ultrastable due to their high thermostability.[15] All the zeolites examined in the present study with the exception of MOR were made up of particles with a rough surface and many small pores, and may have had mesopores among the aggregated particles. Only MOR had a needle-like form and smooth surface, and probably had no mesopores. As markers, we used bovine serum albumin (BSA), cellulase, chymotrypsinogen A, cytochrome C, elastase, hemoglobin, urease, and bovine DNA and RNA.

Table 1 shows adsorption of biopolymers by zeolites at pH 7.5. Proteins with basic pI and chymotrypsinogen A (pI 7.2) bound to all the zeolite species except H-USY, with the lowest Si/Al_2 ratio, and MOR. On the other hand, hemoglobin (pI below 7.0), proteins with acidic pI, and nucleic acids selectively bound to H-USY zeolites irrespective of the Si/Al_2 ratio. Only urease bound to the two MORs and H-FER. These observations suggested that the adsorption of each biopolymer is zeolite-species specific. Some exceptions, however, were also observed (Table 1). The molecular sizes of the intrinsic pores (size ranges less than 1 nm) of zeolites shown in Table 1 are too small to allow the proteins (size range 1–20 nmΦ) to pass or enter, but the external surfaces must be able to adsorb proteins because of their hydrophobicity and ion-exchange capacity. For example, BSA is an oval globule with a long radius of 7 nm. At pH 7.5, Na–Y efficiently adsorbed only proteins with pI values higher than 7.2, such as chymotrypsinogen A, cytochrome C, elastase, and hemoglobin, but showed no adsorption of those with acidic pI values (Table 1). Hemoglobin bound to H-Y but not Na-Y (Table 1). If the adsorption was dependent only on pI of the biopolymers as concluded previously by other researchers,[3–6] chymotrypsinogen A and hemoglobin should have bound to all the zeolites shown in Table 1. However, this was not the case. Adsorption of the biopolymers did not occur only at pI. As described in the Introduction, the conclusions of earlier studies[3–6] were based only on the results of preliminary analysis of the adsorption of a few proteins only to zeolite Y. Therefore, the physicochemical principle underlying adsorption should be systematically investigated by using many species of zeolites and various biopolymers. The candidates of the dominant driving forces for the adsorption of biopolymers include hydrophobic interactions (a kind of van der Waals attraction), ion-exchange of Brönsted acid (Coulombic force) and water substitution at Lewis acid site. When a Lewis acid is bound to a substance such as water, the hydroxyl (OH) group of the water could be substituted to the ionized form when it comes into contact with a stronger base such as an ionized carboxyl base. Proteins may bind to zeolites at the basic side above pI through substitution of the ionized

Table 1. Adsorption of proteins and nucleic acids to various zeolites.[a,b]

Zeolite (Si/Al; pore size[c])	Cytochrome C	Elastase	Chymotrypsinogen A	Hemoglobin	Urease	BSA	Cellulase	DNA	RNA
Na-Y (5.7; 7.4)	+ +	+ +	+ +	− −	− −	− −	− −	− −	− −
H-Y (5.7; 7.4)	+ +	+ +	+ +	+ +	+	−	− −	− −	− −
H-USY (6.3; 7.4)	−	− −	− −	− −	+ +	+ +	+ +	+ +	+ +
H-USY (10.7; 7.4)	+	+ +	+	− −	+ +	+ +	+ +	+ +	+ +
H-USY (13.7; 7.4)	+	+ +	+	+ +	+ +	+ +	+ +	+ +	+ +
Na-BEA (27.4; 7.6 × 6.4, 5.5 × 5.5)	+ +	+ +	+ +	+ +	+	−	−	− −	− −
K-LTL (6.0; 7.1)	+ +	+ +	+ +	− −	− −	− −	− −	− −	− −
K-FER (17.7; 4.2 × 5.4, 3.5 × 4.8)	+ +	+ +	+ +	− −	− −	−	− −	− −	− −
H-FER (19.7; 4.2 × 5.4, 3.5 × 4.8)	+ +	+ +	+ +	+ +	+ +	− −	+	Ø	Ø
H-MOR (15.7; 6.5 × 7.0, 2.6 × 5.7)	− −	− −	− −	− −	+	− −	− −	− −	− −
Na-MOR (15.7; 6.5 × 7.0, 2.6 × 5.7)	− −	− −	− −	− −	+ +	− −	−	Ø	Ø

[a] For details of the proteins and zeolites used, see Experimental Section. [b] − − : 0–25 %; − : 25–50 %; + : 50–75 %; + + : 75–100 %; Ø: not studied. [c] The pore size is given in Å.

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carboxyl base. Since the overall charge of the zeolites themselves is negative, the sum of substitution (attraction) and the negative charge (repulsion) influences binding at the basic side above pI (see Figure 1). Furthermore, the morphology of each of the zeolites and their relationship to the three-dimensional structures of biopolymers should be considered. Since Table 1 suggests a relationship between changes in pH and adsorption, we examined the pH-dependency of adsorption.

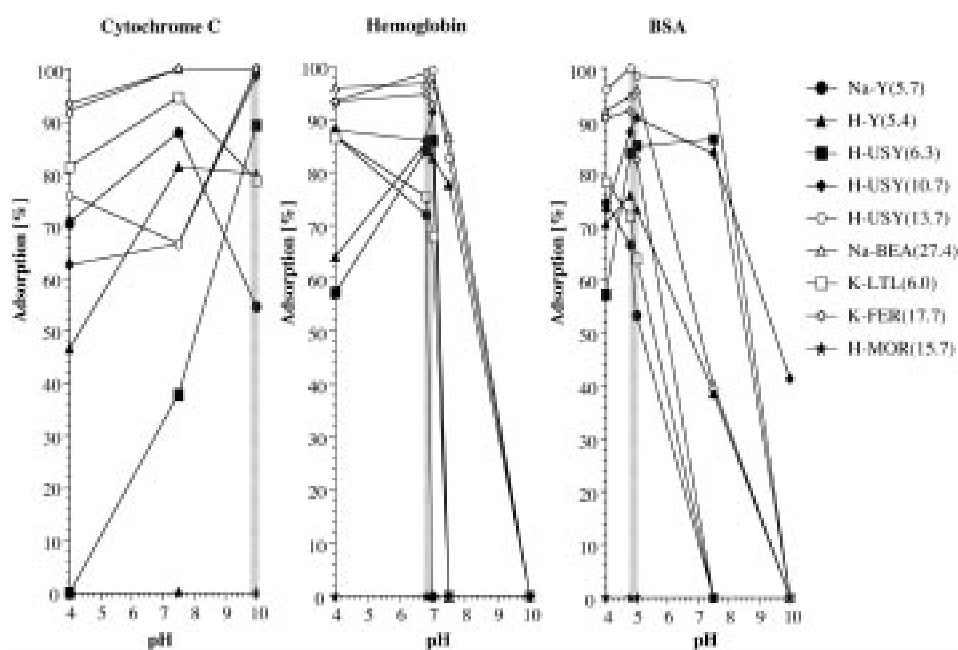

Figure 1. Adsorption of cytochrome C, hemoglobin, and BSA to various zeolites under different pH conditions. Figures in the parentheses after the zeolite names show the ratios of Si/Al_2. The experimental conditions are listed in Table 1 and in the Experimental Section.

Figure 1 shows the adsorption curves of cytochrome C, hemoglobin, and BSA, selected as representative proteins with basic pI, neutral pI and acidic pI, respectively, at various pH values. The maximum adsorption on the zeolites tended to occur when the pH was at or just below the pI of each of the proteins. Some proteins that adsorbed to the zeolites with high Si/Al_2 ratios could bind to the zeolites at a pH above the pI value. The nucleic acids, which have no pI, were adsorbed only to H-USY with a high Si/Al_2 ratio at pH 4 or above (data not shown). The MW of the biopolymers seemed hardly to be related to adsorption. The data shown in Table 1 and Figure 1 indicate that each of the biopolymers tends to bind well at or around its pI to zeolites with higher Si/Al_2 ratio, suggesting that not the ion-exchange capability but the hydrophobicity has the strongest influence on adsorption. These observations, especially the adsorption at pI, partially supported the preliminary results reported by Klint et al.,[6] indicate that protein aggregates that had lost their charge at pI bound to zeolite Y nonspecifically and the adsorption was maximized subsequently. However, protein adsorption also occurred at pH below pI and even at higher pH's the adsorption to some zeolites occurred (see BSA in Figure 1). Aside from the adsorption at pI, with the exception of MOR, most zeolites efficiently adsorbed all the biopolymers at pH below the pI. This could be explained by the negative charge on zeolites. Since proteins have a net negative charge at pH above the pI value and a net positive charge at more acidic pH, the negative charge seems to be a factor that reduces adsorption of protein on zeolite, and a positive charge may be, through cationic ion exchange, a factor that increses adsorp-

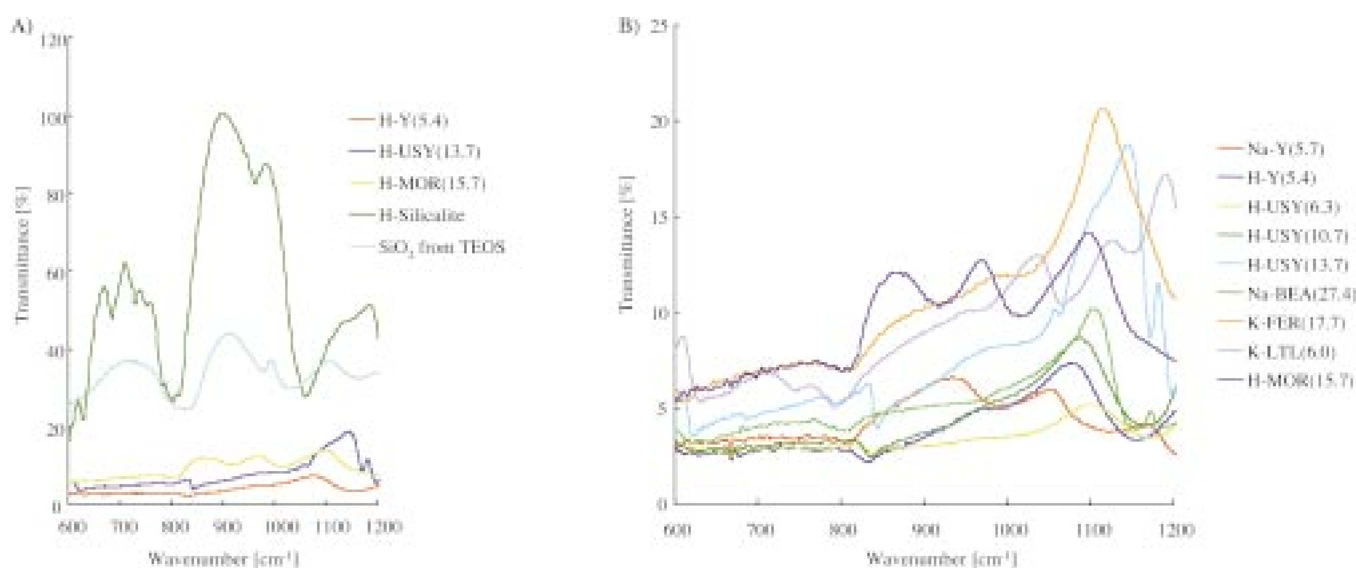

Figure 2. IR spectra of zeolites and silica compounds without Al. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet MAGNA-IR 750 instrument at a resolution of 4 cm^{-1} with 100 scans at room temperature. Figures in the parentheses after the zeolite names show the ratios of Si/Al_2.

tion between the proteins and zeolites. The driving force could be the Coulombic force derived from the positive charges of ionized proteins and the negative charges of the zeolite itself. When Si^{4+} ions in zeolites are partially substituted by Al^{3+} ions, the negative charge could be stronger. This could explain adsorption of biopolymers at pH below the pI (Figure 1). However, protein adsorption at a pH above the pI value, for example, H-USYs and BSA, cannot be explained in this way. Of course, the strongest protein–protein interaction at about the pI of the protein could not be explained by ion exchange.

Figure 2 shows IR spectra of the zeolites used in the present study. The purpose of this experiment was to determine whether the hydroxyl groups on the zeolite surface play a role in adsorption. The signal of the OH group occurs around at 960 cm^{-1} in IR spectrum. As shown in Figure 2, the zeolites that efficiently adsorbed biopolymers had much fewer OH groups; this suggests that the OH groups would have a repulsive effect. These observations indirectly suggest that the charge of zeolites is not necessarily related to adsorption. The interaction between zeolites and biopolymers may be based on both the hydrophobicity and charge of the zeolites, and the strength of the hydrophobic interaction may be reduced in accordance with the charge on the biopolymers. Since proteins with the same net charge repel each other, and since the proteins at pI neutralized the surface charge, adsorption may occur due to hydrophobic interaction, that is, van der Waals attraction, between zeolites and biopolymers.

Zeolites with higher Si/Al_2 ratio and fewer OH groups showed much better adsorption (Figures 1 and 2). Higher Si/Al_2 ratios were associated with a higher degree of hydrophobicity and lower ion-exchange capacity. Adsorption at or above pI is probably related to hydrophobic interaction between zeolites and biopolymers. The reason why the proteins could not bind to zeolites with lower Si/Al_2 ratio and more OH groups is not yet clear. One possibility is that since the proteins could not bind to silicalite, which has no Al, Al atoms in zeolites may play a role in the adsorption. Alternatively, since silicalite has no mesopores (see Figure 3), the number and size of the mesopores may be important for adsorption. The adsorption of biopolymers to zeolites could be considered analogous to salt precipitation in that the dominant driving force is the hydrophobic interaction between the biopolymers and zeolites. If this is indeed the case partitioning would be similar to that in hydrophobic chromatography, in which proteins are bound to fatty-acid-agarose gel in the presence of high salt concentration and then eluted by reducing the salt level. However, all the biopolymers used could bind to zeolites in the absence of salt and could be eluted by increasing the salt levels (data not shown). These results did not support the above suggestion. Moreover, the observation that zeolites adsorbed the biopolymers most efficiently at their pI values without salts is markedly different from the case of hydrophobic chromatography.

As shown in Table 1 and Figure 1, there was one exception in that MOR, one of the zeolites with high Si/Al_2 ratio and few OH groups, was not capable of adsorbing biopolymers. The morphological structure of MOR is markedly different from those of the other zeolites with have high Si/Al_2 ratio and few OH groups such as USYs. MOR has a needle-like form with a smooth surface, and intrinsic pores are present at both sides of the needle edge. The other zeolites with high Si/Al_2 ratio and few OH groups are particles with a rough surface and tend to form aggregates. As described above, the so-called mesopores form between the aggregated particles. Only MOR does not have mesopores because of its smooth surface. If adsorption occurs mainly by hydrophobic interaction, the surface structure is important. Since the hydrophobic interaction is thought to be much weaker for binding than Coulombic force (about one eight hundredth in vacuum, and about one thirtieth in water),[16] the binding between zeolite and biopolymer must require many van der Waals force points on both sides of the bound surfaces. The adsorption at pI may greatly depend on the surface structure, especially the mesopores, and its relation to the hydrophobic interaction, because the adsorption and repulsion mediated by the Coulombic force does not occur at the pI point. The results reported here suggest that the Coulombic force may disturb the hydrophobic interaction on the zeolite surface.

We next measured the mesopores of zeolites such as Y, USY, BEA, LTL, FER, and MOR. Figure 3 shows the determined radii (Rp) of the mesopores of zeolites. Many of the zeolites possess mesopores of over 2 nm, and, in particular, USYs and FER have large mesopores in the range of 5–40 nm (Figure 3). Interestingly, the zeolites in Table 1 that efficiently adsorbed the biopolymers tended to have relatively large mesopores in the range of 5–40 nm, but those with mesopores less than 2 nm showed less efficient binding. Comparison of the two Ys (Si/Al_2 ratio 5.4 and 5.7) and three USYs (Si/Al_2 ratio 6.3, 10.7, and 13.7) suggest that zeolites with high Si/Al_2 ratio are likely to form larger numbers of mesopores with larger radii. USY (13.7), which showed the most efficient adsorption, had many mesopores with a radius >10 nm (Figure 3). A pore radius size of 5 to 10 nm is sufficient to hold most of the proteins used here (range 0.5 to 5 nm). Although urease and nucleic acids are larger than 10 nm, their stringlike structures should be taken into consideration. Under conditions in which adsorption and repulsion mediated by the Coulombic force does not occur, hydrophobic interactions may occur at many points on the surface of the mesopores by surface-to-surface interaction resulting in adsorption of the biopolymers. On the other hand, since the proteins shrink at pI due to loss of their surface charge, they would be more readily adsorbed into the mesopores. At pI, the binding to zeolites can be considered analogous to neutralized precipitation between the biopolymer molecules and zeolites, in that the dominant driving force is the hydrophobic interaction with the zeolite mesopores.

Above pI, some zeolites such as H-USYs adsorbed the biopolymers efficiently, although the repulsion mediated Coulombic force occurs and the biopolymers are active and swollen. This phenomenon, therefore, could not be explained only by the hydrophobic interaction and the mesopores. In this case, the dominant driving force might be the water substitution reaction on Al of the zeolite surface. Since it is well known that carboxylate anions can easily be substituted for water coordinating to metal ions, the negative charges on the biopolymers above pI may cause substitution of water on

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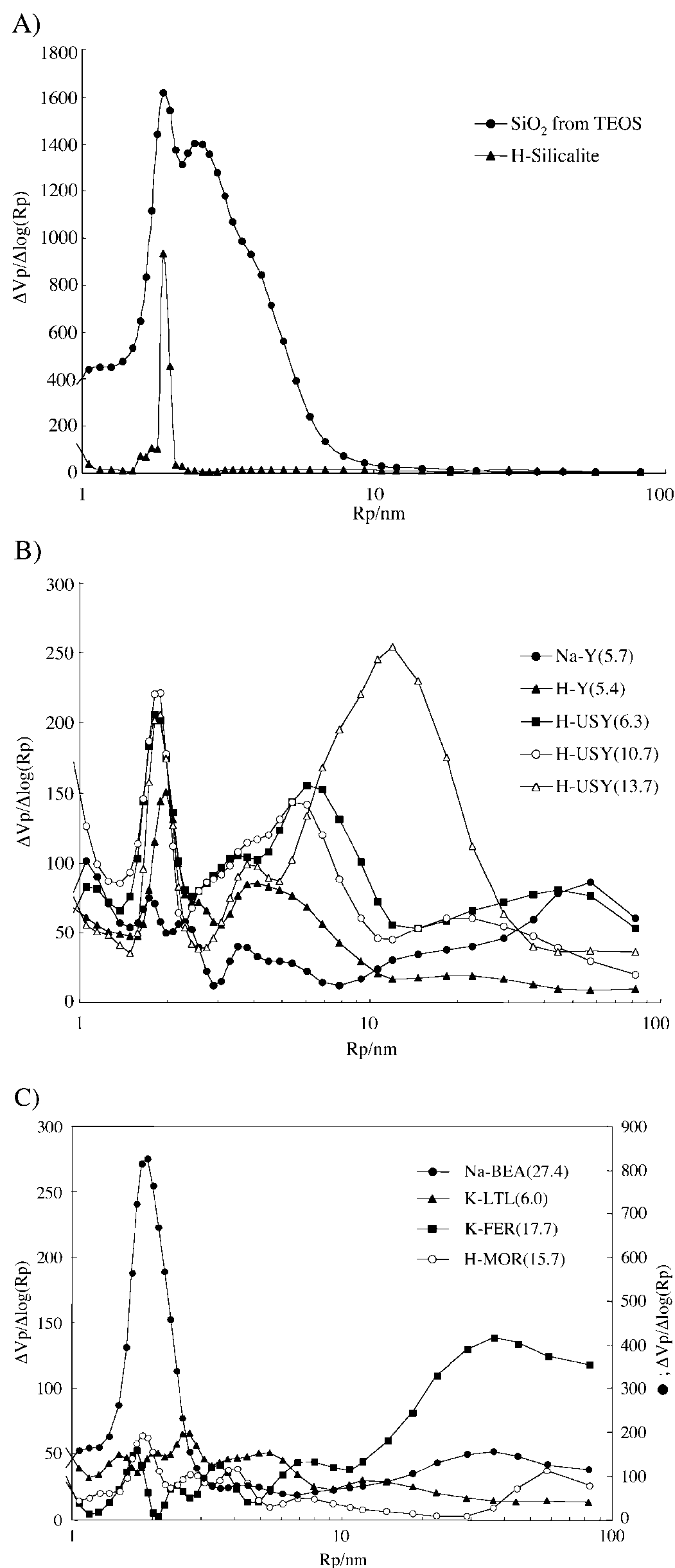

Figure 3. Size and distribution of pores in zeolites. The adsorption and desorption isotherms of N_2 were measured using a BELSORP 28 instrument. The pore size distribution was calculated according to the D-H method from the desorption branch. Rp = radius of the pore. ΔVp/Δlog(Rp) = differential pore volume A) Silica compounds without Al: SiO_2 from TEOS (amorphous silica) and H-Silicalite. B) Zeolite Y: Na-Y (Si/Al_2 ratio, 5.7), H-Y (Si/Al_2 ratio, 5.4), H-USY (Si/Al_2 ratio, 6.3), H-USY (Si/Al_2 ratio, 10.7) and H-USY (Si/Al_2 ratio, 13.7). C) Other zeolites: Na-BEA (Si/Al_2 ratio, 27.4), K-LTL (Si/Al_2 ratio, 6.0), K-FER (Si/Al_2 ratio, 17.7) and H-MOR (Si/Al_2 ratio, 15.7).

Lewis acid sites of Al, resulting in efficient polymer binding to H-USY, but not to most of the other zeolites, which have fewer Lewis acid points on their surface.

In conclusion, zeolites selectively adsorbed biopolymers on their surface; this may be as a result of the following factors: 1) below pI, mainly the Coulombic attraction similar to ion-exchange chromatography; 2) at pI, probably hydrophobic interactions and the mesopore structure; and 3) above pI, hydrophobic interactions and substitution of water at the Lewis acid sites of Al. When the Si/Al_2 ratio is high, but Al level low, and in the presence of mesopores between the zeolite particles the adsorption was maximal at pI; this suggests that the adsorption is markedly dependent on the number of hydrophobic interaction points on the mesopores and on their morphology.

Preliminary reports[3–5] indicated that under high salt conditions proteins conformed to the expected behavior typical of hydrophobic interaction, that is, increased binding with increasing salt concentrations, but at low salt concentrations the binding first underwent a decrease. The charge-mediated repulsion is quenched by ions, and excess adsorption occurred as a result of less strong hydrophobic interactions, which can be altered by a change in pH, was removed beforehand by adjusting the ion composition of the solution. This explanation appeared to be applicable to the zeolite Ys based on the present results, but not for the other zeolites used here (Table 1 and Figure 2). Most of the zeolites adsorbed biopolymers in the absence of high salt concentration, and some showed good adsorption regardless of pH. Previously, it was concluded, that adsorption occurred at the isoelectric point (pI) due to a loss of charge of the protein aggregate.[6] However, in the presence of 8 M urea, a concentration that is sufficient to solubilize the aggregate at the pI, most of the zeolites were still able to adsorb the biopolymers (data not shown). In the earlier report,[6] no reasonable explanation for this phenomenon was given, and the results of the present study obtained with various zeolites suggest that another interpretation of their findings is required. Our explanation outlined above is more suitable.

As described here, some zeolites could selectively adsorb proteins and nucleic acids at their pI and may release them under special conditions. Zeolites could be used for the purification of proteins according to different physicochemical principles as used in standard chromatographic procedures. The adsorption of biopolymers, including nucleic acids, may provide preliminary information regarding whether conjugated proteins could be directly fractionated in future experiments. Many biological structural matrices, for example, chromosomes and membranes, that contain conjugated proteins are lost when precipitated or have a reduced net charge. Even cells are caused to precipitate by the net surface charge. This makes it possible to utilize zeolites in the purification of conjugated proteins, the structural matrix, and even cells in a differential state.

Experimental Section

Zeolite Y, zeolite USY, zeolite beta (Na-BEA, HSZ-930NHA, Si/Al_2 ratio is 27.4), ferrierite (K-FER, HSZ-720KOA), zeolite L (K-LTL, HSZ-

500 KOA) and mordenite (H-MOR, HSZ-620HOA) were obtained from Tosoh (Japan). Zeolite Y included proton-type (H-Y, HSZ-320HOA, Si/Al_2 ratio 5.7) and Na-type (Na-Y, HSZ-320NAA, Si/Al_2 ratio 5.4). Zeolite USY (H-USY, HSZ-330,350,360HUA) included three species with different Si/Al_2 ratios (6.3, 10.7, and 13.7). Zeolite FER and MOR both included two species, K-FER (Si/Al_2 ratio, 17.7) and H-FER (Si/Al_2 ratio, 17.7), and H-MOR (Si/Al_2 ratio, 15.7) and Na-MOR (Si/Al_2 ratio, 15.7), respectively. The proteins used as markers were bovine serum albumin (BSA, MW 69 kDa, pI 4.9), cellulase (MW 35 kDa, pI 3.7), chymotrypsinogen A (MW 25 kDa, pI 7.2), cytochrome C (MW 12.4 kDa, pI 10.1), elastase (MW 25.9 kDa, pI 9.5), hemoglobin (MW 65 kDa, pI 6.8–7.0) and urease (MW 480 kDa, pI 5.0–5.1), and were purchased from Wako Pure Chemical Industries (Japan). Nucleic acids, that is, DNA from calf thymus and RNA from calf liver, were purchased from Sigma (St. Louis, MO).

All the zeolites used in this study were shown to have reagent grade purity and crystallinity by X-ray powder diffraction. The average particle size and size range of zeolites were 0.3 μm and 0.3–15 μm, respectively, as estimated from scanning electron microscopy images. The size of the primary crystals was 0.3 μm, and some big crystal particles were aggregates that consisted of small primary crystals.

Ion exchange of zeolites (K-FER to H-FER and H-MOR to Na-MOR) was performed as follows. K-FER and H-MOR were suspended in 0.5 M NH_4NO_3 and 0.5 M $NaNO_3$, respectively. After stirring for 6 h, both samples were filtered and washed with distilled water. Finally, samples were dried at 50 °C for 20 h and calcined at 400 °C for 20 h. Adsorption of proteins and nucleic acids was carried out by incubation of each suspension (proteins; 3 $mg\,mL^{-1}$, nucleic acids; 250 $\mu g\,mL^{-1}$) with zeolites (100 mg). Zeolites were suspended in of Tris-EDTA buffer (1 mL; 10 mM Tris-HCl (pH 7.5)/1 mM EDTA), degassed and centrifuged at 10000 rpm. Then, aliquots of 500 μL of the supernatant were removed, and 500 μL of each suspension (proteins; 6 $mg\,mL^{-1}$, nucleic acids; 500 $\mu g\,mL^{-1}$) was added. Incubation was carried out for 1 h on a ROTARY CULTURE RCC-100 (IWAKI GLASS) at room temperature, because the adsorption equilibrium was reached around 0.5 h after mixing. Supernatants were obtained by centrifugation at 12000 rpm (twice). Absorbance was determined at 595 nm (except for cellulase; 280 nm) and 260 nm to calculate the amounts of proteins and nucleic acids adsorbed to zeolites. The following buffers were used: pH 4.0, acetate buffer [acetic acid (82 mM) /sodium acetate (18 mM) ($CH_3COONa/3H_2O$)]; pH 4.8, acetate buffer [acetic acid (40 mM)/sodium acetate (60 mM) ($CH_3COONa/3H_2O$)]; pH 5.0, acetate buffer [acetic acid (29.6 mM)/sodium acetate (70.4 mM) ($CH_3COONa/3H_2O$)]; pH 6.8, MOPS [MOPS (78 mM)/NaOH (22 mM)]; pH 7.0, MOPS [MOPS (71 mM)/NaOH (29 mM)]; pH 10.0, glycine–NaOH buffer [glycine (50 mM)/NaOH (32 mM)].

[1] F. Mizukami, H. Izutsu, T. Osaka, Y. Akiyama, N. Uiji, K. Moriya, K. Endo, K. Maeda, Y. Kiyozumi, K. Sakaguchi, *J. Chromatogr. A* **1995**, *697*, 279–287.

[2] Y. Akiyama, F. Mizukami, Y. Kiyozumi, K. Maeda, H. Izutsu, K. Sakaguchi, *Angew. Chem.* **1999**, *111*, 1510–1513; *Angew. Chem. Int. Ed.* **1999**, *38*, 1420–1422.

[3] S. Ghose, B. Mattiasson, *Biotechnol. Appl. Biochem.* **1993**, *18*, 311–320.

[4] D. Klint, P. Arvidsson, Z. Blum, H. Eriksson, *Protein Expression Purif.* **1994**, *5*, 569–576.

[5] D. Klint, H. Eriksson, *Protein Expression Purif.* **1997**, *10*, 247–255.

[6] D. Klint, G. Karsson, J.-O. Bovin, *Angew. Chem.* **1999**, *111*, 2736–2738; *Angew. Chem. Int. Ed.* **1999**, *38*, 2560–2562.

[7] M. E. Davis, R. F. Lobo, *Chem. Mater.* **1992**, *4*, 756–768.

[8] H. Kessler in *Comprehensive Supramolecular Chemistry*, *Vol. 7* (Eds.: G. Alberti, T. Bein, J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, J.-M. Lehn), Pergamon, Oxford, **1996**, pp. 425–464.

[9] S. L. Burkett, M. E. Davis in *Comprehensive Supramolecular Chemistry*, *Vol. 7* (Eds.: G. Alberti, T. Bein, J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, J.-M. Lehn), Pergamon, Oxford, **1996**, pp. 465–483.

[10] D. W. Breck, *Zeolite Molecular Sieves*, Wiley, London, **1974**.

[11] R. Szostak in *Modified Zeolites; Introduction to Zeolite Science and Practice, Studies in Surface Science and Catalysis*, *Vol. 58* (Eds.: H. van Bekkum, E. M. Flanigen, J. C. Jansen), Elsevier, Amsterdam, **1991**, Chapter 5.

[12] W. M. Meier, D. H. Olson, C. Baerlocher, *Atlas of Zeolite Structure Types*, Elsevier, London, **1996**.

[13] a) S. Feng, T. Bein, *Nature* **1994**, *368*, 834–836; b) S. Feng, T. Bein, *Science* **1994**, *265*, 1839–1841; c) Y. Yan, T. Bein, *J. Am. Chem. Soc.* **1995**, *117*, 9990; d) S. Mintova, B. J. Schoeman, V. Valtchev, J. Sterte, S. Mo, T. Bein, *Adv. Mater.* **1997**, *9*, 585; e) G. A. Ozin, *Adv. Mater.* **1992**, *4*, 612; f) P. Behrens, G. A. Stucky in *Comprehensive Supramolecular Chemistry*, *Vol. 7* (Eds.: G. Alberti, T. Bein, J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, J.-M. Lehn), Pergamon, Oxford, **1996**, pp. 721–772; g) T. Bein, *Chem. Mater.* **1996**, *8*, 1636.

[14] S. Mintova, N. H. Olson, V. Valtchev, T. Bein, *Science* **1999**, *283*, 958–960.

[15] J. M. Newsam in *Solid-State Chemistry*: *Compounds* (Eds.: A. K. Cheetham, P. Day), Clarendon, Oxford, **1992**, Chapter 7.

[16] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson in *Molecular Biology of the Cell*, 2nd ed. (Eds.: B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson), Garland, NY, **1989**, p. 89.

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